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Antimicrobial Activity of Essential Oil from *Tagetes minuta* L. and its Extracts along with its GC-MS Profiling

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Abstract

Nepal, being a land of diverse topography with the different weather conditions, is considered as storehouse of medicinal and aromatic plants (MAPs) with enriches medicinal values. *Tagetes minuta* L. is highly demanded aromatic plant for its essential oil, hugely used in flavor and perfumery industries, having potential bioactive and therapeutic properties. The essential oil was extracted from aerial parts of *Tagetes minuta L.* via hydro-distillation and its chemical composition was analyzed using GC-MS technique. Furthermore, the antimicrobial activity of oil and its hexane, ethylacetate, and methanolic extracts was assessed against different pathogenic bacterial strains using agar well diffusion method. The essential oil yield was 0.3% (v/w) and GC-MS data revealed the highest percentage of monoterpenes: alpha pinene (33.12 %), transocimenone (23.32 %), cistagetone (12.18%) and dihydrotagetone (9.65%) along with other hydrocarbons. Similarly, the antimicrobial activity showed ethylacetate and methanolic extracts to be quite active against pathogenic bacterial strains *Staphylococcus aureus* (ATCC 6538P) with Zones of Inhibition (ZOI) 14.30 mm and 10.40 mm and *Staphylococcus epidermidis* (ATCC 12228) with ZOI value of 17.50 mm and 16.30 mm respectively. On the other hand, it also showed moderate activity against pathogenic bacterial strains *Bacillus subtilis* (ATCC 8739) and *Proteus vulgaris* (ATCC 6380*)*. Although additional necessary investigations are needed, these results support the use of traditional medicinal plants in treating various diseases. Also, these results suggest that the studied essential oil may have potential for isolating the bioactive compounds, which could contribute to the discovery of noble drugs in future.

Keywords: Medicinal and aromatic plants, Monoterpenes , α-pinene, *Tagetes minuta* L. essential oil

Introduction

Nepal is ranked as 9th among the Asian countries for its floral wealth with an estimated 9,000 species of flowering plants (Kunwar et al., 2023). Among which, it is supposed that 1600-1900 species are medicinal and aromatic plants commonly used for the medicinal purpose (Ghimire et al., 2008).

Essential oils (EOs), also referred as ethereal or volatile oils, have been used for extensive applications in pharmaceutical, medical, and perfume industries. They are aromatic oily liquids obtained from different parts of plant, i.e., leaves, seeds, fruits, buds, flowers, herbs, barks, and roots (Silori et al., 2019). Similarly, they are natural extracts of aromatic plants used in many fields like agriculture, aromatherapy and nutrition (Bolouri et al., 2022). EOs are a complex mixture of mainly terpenes particularly monoterpenes and sesquiterpenes and their oxygenated derivatives such as alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides (Badawy & Abdelgaleil, 2014). The pharmacological studies suggest that these EOs can be used as anti-rheumatic, antiseptic, antispasmodic, anticancer, anti-inflammatory, antitoxic, aphrodisiac, and astringent agents (Bhardwaj et al., 2020).

Tagetes minuta L. is annual, strongly aromatic herb of sunflower family (Asteraceae), one of the most abundant plants taxonomical grouping, comprising about 1,000 genera and over 23,000 species (Sadia et al., 2013).The genus *Tagetes* refers to the Latin name for marigold '*Tages*', an Etruscan god associated with agriculture and the species *T. minuta* is from the Latin word '*minutes*' meaning small referring to its small sized capitula (Bandana et al., 2018). The stem is 1-2 meter tall and branched with 5-15 cm long opposite leaves divided into one terminal and several (3-7) lateral leaflets. Flowers are creamy

yellow in color and blooms from September to December in late summer (Hulina, 2008). This plant has been initially originated from South America and found in temperate grasslands and elevated regions.

In the context of Nepal, *T. minuta* has been listed as an medicinal plants prioritized for economic development (Department of Plant Resources [DPR], 2024). This plant thrives particularly in western Himalayan region and has gained popularity among farmers for its essential oil production and aroma industries (Walia & Kumar, 2020). This plant exhibits moderate tolerance to various soil conditions, making it suitable for diverse agricultural environments in Nepal. Similarly, its resilience to adverse conditions, such as high salinity and pH levels, enhances its potential as a sustainable crop in challenging terrains (Sahay & Patra, 2013). Walia et al. (2020) performed a recent study on variations in essential oil composition of *T. minuta* L. provided the meaningful information for diversified phytotherapy and for improved utilization in food, flavor and fragrance industries. Extensive studies on the phytochemistry and pharmacology of *T. minuta* have identified the presence of essential oils, thiophenes, flavonoids, terpenes, saponins, carotenoids, and other chemical compounds in this species (Verma et al., 2024). Meshkatalsadat et al. (2010) conducted a research on the chemical characterization of volatile components of *T. minuta* cultivated in south west of Iran by nano scale injection technique confirmed the presence of more than 27 compounds that are mainly limonene, piperitenone, α-terpinolene, piperitone, (E)-tagetone, (Z)-ocimenone and so on. Similarly, another research performed by Singh et al. (2016) identified the major chemical constituents to be Z-β-ocimene, limonene, dihydrotagetone, tagetones (E&Z) and ocimenones (E & Z).

In recent years, a wide range of plant essential oils and their constituents have been investigated for their antibacterial properties against an array of plant pathogenic bacteria (Gakuubi et al., 2016) and they have also several medicinal benefits, which include remedy for colds, respiratory inflammations and stomach problems (Karimian et al., 2014a). Another research emphasized naturalized exotic weed, *Tagetes minuta* as a potentially invasive species in Nepal (Lamichhane et al., 2025). Some previous studies performed in Nepal on this plant have highlighted its antimicrobial properties as well as its ethno-medicinal values (Khakurel et al., 2014). Although a few articles on the antimicrobial studies of essential oils obtained from this plant have previously been reported internationally (Senatore et al., 2004) and nationally (Joshi et al., 2014), the antimicrobial activities of various Soxhlet extracts have not yet been thoroughly explored, particularly in the context of Nepal. The major aim of the present study was to evaluate the antimicrobial activities of hexane, ethyl acetate and methanolic extracts of *T. minuta* as well as its essential oil, against different pathogenic bacterial and fungi strains.

Materials and Methods

Sample collection

The fresh plants of *T. minuta* (collection no. M003) were collected from Marpha of Mustang district in the month of October, 2023. The locality where the plant material was collected was nearly at the altitude of 2700 meter above the sea level. The plant material was identified by National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur with the voucher code no. KATH170291.

Essential oil extraction

Essential oil from the shade-dried plant sample was extracted by hydro-distillation method. Small pieces of aerial parts of the plant materials were tightly packed in round bottom flask and fitted to a Clevenger apparatus and condenser. The oil was extracted for about 4 hours and was collected in glass vial. The moisture present in the essential oil was later removed by using anhydrous sodium sulphate and the obtained oil was stored under refrigerated conditions (Walia et al., 2020).

The essential oil content (v/w) % was calculated using the formula:

$$\text{Essential oil content (\%)} = \frac{E_1}{E_2} \times 100$$

Where, E_1= Essential oil volume (mL),
E_2= Fresh sample weight (gm)

Identification of compounds of essential oil by GC-MS

The essential oil obtained from *T. minuta* was dissolved in solvent forming 1:10 ratio i.e. the sample solution was prepared in a sample vial by dissolving 100 µL of essential oil in 1000 µL hexane as solvent, sonicating for 5-10 minutes and then used for further process.

Compounds of *T. minuta* essential oil were analyzed by Shimadzu GC-MS-QP 2010 Plus available at the Instrument Section of the Department of Plant Resources. The capillary column used for the analysis was SH-RTX-5MS (60 m _ 0.32 mm _ 0.25 m) with a crossbond of 5% diphenyl / 95% dimethyl polysiloxane as the stationary phase. The GC analysis was performed under the following conditions: column oven temperature - 50°C, injection temperature - 250°C, ion source temperature - 250°C, interface temperature - 200°C, split injection mode with a split ratio of 80, electron impact (EI) mode - 70 eV, ion source temperature - 250°C, Helium with a pressure of 53.8 kPa, total gas flow - 112.3 mL/min and column flow - 1.35 mL/min. The GC-MS system starts with an initial oven temperature of 50°C for 1 minute, and then increases to 230°C at a rate of 3°C for 9 minutes. Mass spectral detection was carried out in electron ionization mode by scanning at 40 to 350 m/z. The total time required for analyzing a single sample was 70 min (Pradhan et al., 2023).

The chemical components of the essential oils were identified by comparing their mass spectral fragmentation patterns with those in the National Institute of Standard Technology Library (NIST) 2017 and Flavor and Fragrance Natural and Synthetic Compounds FFNSC 4.0 library search facility linked with the data analysis software of the GC-MS (Adams, 2005). The percentage of each component (Area %) is reported as raw percentages based on the total ion chromatogram (TIC) without standardization.

Soxhlet extraction method

The crude sample was placed in a thimble-shaped filter paper which is then kept in a glass cylinder provided with a siphon tube and an inlet tube. A water condenser was attached to the cylinder at the top and the entire assembly was fitted into the neck of a round bottom flask containing the solvent. The flask was heated in water bath. The solvent vapors reached the cylinder through the inlet tube and condensed on passing upward into the condenser. The condensed solvent came in contact with the crude organic substance and dissolved it as soon as the solution reached the top end of the siphon tube. In this way, a continuous supply of solvent vapors was maintained in the cylinder, and the dissolved organic compound flowed back into the flask. Finally, the heating was stopped and the solution in the flask was distilled to recover the solvent, while the organic compound left behind was collected as the form of sample extract.

Here, the Hexane (non-polar), ethylacetate (moderately polar) and methanol (polar) solvents were consecutively used as the solvents (Abubakar & Haque, 2020).

Antimicrobial activity test by agar well diffusion method

The antimicrobial activity of essential oils of *T. minuta* was evaluated by agar well diffusion method (AWD) as described by Clinical and Laboratory Standard Institute (CLSI) guidelines (Cockerill & CLSI, 2012). The pathogenicity of the bacterial isolates were not checked and the used bacterial strains (Microbiologics, Cooper Avenue North, Saint cloud, Minnesota, USA) were of: *Bacillus subtilis* (ATCC 6059), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 8739), *Klebsiella quasipneumonia* (ATCC 700603), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella entericatyphi* (clinical sample), *Shigella dysenteriae* (clinical sample), *Staphylococcus aureus* (ATCC 6538P) and *Staphylococcus epidermidis* (ATCC 12228) and a fungi *Candida albicans* (ATCC 10231).

The bacterial and fungal strains were cultured in nutrient agar and incubated at 35 °C in incubator (Accumax Equipments, India) for growth. The inoculum suspension was prepared in normal saline solution and was spread uniformly with a sterile cotton swab on Mueller Hinton Agar (HiMedia, India) plates. A 6 mm diameter hole was aseptically punched on the agar surface with a sterile cork borer, and 50 µL of the essential oil/sample extract were introduced into each well. Ciprofloxacin (CIP 5 mcg/disc) were used as positive control for tested bacterial strains- *B. subtilis, E. faecalis, E. coli, K. quasipneumoniae, P. vulgaris, P. aeruginosa, S. typhi and S. dysenteriae,* Amoxycillin (AMX 10 mcg/disc) for *S. aureus and S. epidermidis* and Clotrimazole (CL 10 mcg/disc) for fungi *C. albicans* respectively. Similarly, dimethyl sulfoxide (DMSO) was used as negative control. The plates were kept for 2-3 hours to allow diffusion of essential oils into the agar medium and incubated at 35°C for 24 hours. In this way, antimicrobial assay was performed and zone of inhibition were measured by using vernier caliper. The sensitivity was categorized as: not sensitive (diameter ≤8 mm); sensitive (diameter 9-14 mm); very sensitive (≥15 mm) (Ponce et al., 2003).

Results and Discussion

Essential oil content and composition

A yellow colored essential oil was obtained from the fresh biomass of *Tagetes minuta* L. yielding 0.3% (v/w) based on their fresh weight. Previous studies of essential oil from four *Tagetes* species collected in Hungary revealed the oil percentage ranged between (0.5-1.18)% (Hethelyi, et al., 1986). Bahadirli (2020) reported that 1.8% of essential oil was isolated from *Tagetes minuta* from Turkey. Similarly, Walia et al. (2020) reported that the essential oil content of fresh *T. minuta* plant varied from 0.37 to 0.79% across different altitudinal locations.

The chemical composition of the essential oil was analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS), which enabled the identification of several major constituents contributing predominantly to the total oil content. GC-MS analysis of the essential oil led to the identification of over 20 distinct volatile compounds in the essential oil. Among these, α-pinene exhibited the highest relative abundance, accounting for 33.12% of the total peak area, followed by trans-ocimenone (23.32%). Other prominent constituents included cis-tagetone (12.18%), dihydrotagetone (9.65%), and cis-ocimenone (7.91%). The percentage composition of monoterpene hydrocarbon, dihydrotagetone, ocimenone is found to be comparable with previous study reported by (Babaei et al., 2021). Their research revealed that water limitation had a considerable effect on the essential oil composition of *T. minuta*. Results showed that out of 30 compounds, the essential oil mainly consisted of oxygenated monoterpenes (65.3-75.3%), which represented with dihydrotagetone (35.8-40.9%) as the major component, followed by *E*-(Z)-tagetone (15.4-17.6%). Monoterpene hydrocarbons (20.6-27.2%) represented by β-ocimene (11.6-18.5%) were also identified along with other components (E)-ocimenone, (Z)-ocimenone, limonene and so on. GC-MS analysis of essential oil of *T.minuta* L. revealed a greatly diversified proportions of compounds rich in many secondary compounds, including monocyclic and bicyclic monoterpenes (47.90%), sesquiterpenes (30.20%), hemiterpenes (15.13%) and diterpenes (1.68%), confirmed from the previous findings by Wanzala & Ogoma (2013). Their extensive research highlighted the higher proportion of monoterpene constituents of *Tagetes* oil compared favourably with the results obtained from this report. The results from this study and those from other literature further confirmsthat the main principal constituents of the essential oil of *T. minuta* are ocimene, dihydrotagetone, tagetones and ocimenones. These compounds form the basis of using this oil in the pharmaceutical, agricultural, food and perfumery industries, contributing its high demand (Singh et al., 2003).

α-Pinene is a bicyclic monoterpene widely found naturally as an insect-repellent agent in plant defense (Huang et al., 2013). When α- and β-pinenes are the major constituents of an essential oil, they warrant the anti-inflammatory and analgesic activity (Mercier et al., 2009). Similarly, ocimenone, an unsaturated

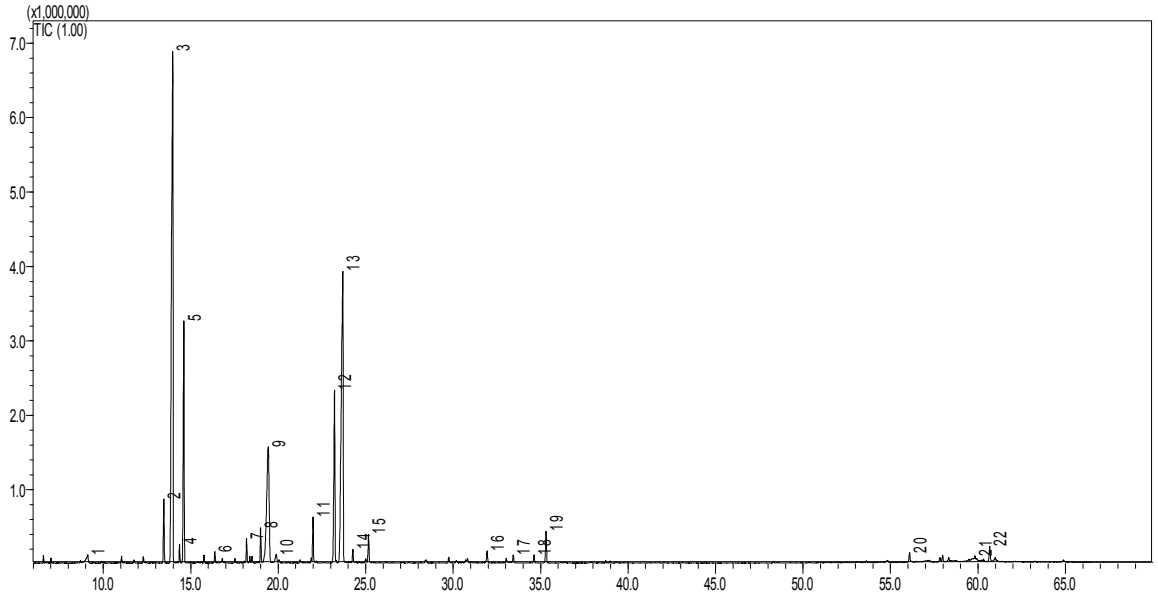


Figure 1: Chromatogram of *Tagetes minuta* L. essential oil representing Retention time (min) in X-axis vs Intensity (TIC * 1,000,000) in Y-axis

acylicmonoterpene hydrocarbon and tagetone and dihydrotagetone, oxygenated monoterpene, are also present abundantly in *T. minuta* essential oil similar as reported from Kyarimpa et al. (2015) as well as Rathore et al. (2018). Extensive studies on the phytochemistry has revealed the presence of various phytoconstituents such as thiophenes, flavonoids, terpenes, saponins, carotenoids, and other chemical compounds in its essential oil. These phytoconstituents contribute to various pharmacological activities, including antimicrobial (Al-Robai et al., 2023), anticancer (Oyenihi et al., 2021), antidiabetic (Lim et al., 2023), antioxidant and anti-inflammatory properties (Karimian et al., 2014b) etc.

The GC-MS chromatogram and the corresponding total ion chromatogram (TIC) profile are presented in (Figure 1), illustrating the separation and relative abundance of the detected compounds. The components, identified based on their mass spectral data, and retention time are listed in (Table 1).

Table 1: Chemical composition of *Tagetes minuta* L. essential oil

Peak	Retention Time (min)	Area %	Name
1.	9.116	0.8	3,3,5-Trimethylcyclohexene
2.	13.462	2.13	Limonene
3.	13.977	33.12	α-Pinene
4.	14.359	0.58	(E)-β-Ocimene
5.	14.61	9.65	Dihydrotagetone
6.	16.38	0.38	Carvenone
7.	18.193	0.84	neo-allo-Ocimene
8.	18.994	1.23	trans-Tagetone
9.	19.428	12.18	cis-Tagetone
10.	19.884	0.64	2-allylfuran-3-carboxylate
11.	21.99	1.65	4-methyleneisophorone
12.	23.221	7.91	cis-Ocimenone
13.	23.695	23.32	trans-Ocimenone
14.	24.268	0.48	Carvacrol
15.	25.162	1.11	Livacone
16.	31.938	0.56	(E)-Caryophyllene
17.	33.435	0.34	α-Humulene
18.	34.623	0.31	γ-Cadinene
19.	35.31	1.3	Bicyclogermacrene
20.	56.086	0.47	trans-α-Atlantone
21.	59.822	0.36	Adamantan-2-one
22.	60.668	0.66	Artemisia ketone

Antimicrobial activity

The results on antimicrobial activity test of essential oil of *T. minuta* against different pathogenic bacterial strains are tabulated in (Table 2). The essential oil of *T. minuta* demonstrated promising antimicrobial activity against the gram-negative bacterial strain *Proteus vulgaris*, and the gram positive bacterial strains *Staphylococcus aureus* and *Staphylococcus epidermidis,* showing significant zone of inhibition of 10.50 mm, 12.30 mm and

11.20 mm respectively. This can be interpreted as it was moderately active as compared to the positive controls used i.e. Ciprofloxacin with zone of inhibition 32.06 mm and Amoxycillin with ZOI 35.10 mm and 17.74 mm. Senatore et.al. (2004) evaluated the antimicrobial activity of essential oil of *T. minuta* against different gram-positive and gram-negative bacterial strains using broth dilution method. Their findings suggested that UK (United Kingdom) based sample oil was more active than the South African samples against all the bacteria tested and more active than the Egyptian oil against all bacterial strains except the gram-negative *Proteus mirabilis*, against which it showed the same activity. Gakuubi et al. (2006) revealed that the essential oils of *T. minuta* showed promising antibacterial activities against the test pathogens with *Pseudomonas savastanoi* pv. *Phaseolicola* among others (*Xanthomonas axonopodis* pv. *Phaseol* and *Xanthomonas axonopodis* pv. *manihotis*) being the most susceptible with mean inhibition zone diameters of 41.83 mm and 44.83 mm after 24 and 48 hours, respectively. The minimum inhibitory concentrations and minimum bactericidal concentrations of the EOs on the test bacteria were in the ranges of 24-48 mg/mL and 95-190 mg/mL, respectively. Similarly, Walia et al. (2020) investigated the antimicrobial activity against two gram-positive bacteria viz. *Micrococcus luteus*, and *Staphylococcus aureus*, and two gram-negative bacteria viz. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using agar well-diffusion and micro-dilution methods. The agar well diffusion method demonstrated stronger activity of *T. minuta* EOs in gram positive bacteria as compared to gram negative bacteria. The highest activity was observed against *S. aureus* with zone of inhibition above 9 mm. The most potential EOs (three locations of Himalchal Pradesh and one location of Manipur) demonstrated an MIC of 25-30% (v/v). According to Trombetta et al. (2005), gram-positive bacterial strains are usually more susceptible to the antibacterial activity of lipophilic essential oils because of the lipophilic nature of the gram-positive cell membrane, as compared to the hydrophilic character of the cell membrane component of gram-negative bacteria.

Different Soxhlet extracts of *T. minuta* exhibited some antimicrobial activity against various tested bacterial strains as tabulated in (Table 3). Among the tested three extracts, hexane extract did not show any activity against all the tested microbes, but ethyl acetate and methanolic extracts showed significant activity against different bacterial strains. Hexane, being a non-polar solvent does not efficiently extract polar or semi-polar phytochemicals such as

Table 2: Antimicrobial activity test of essential oil of *T. minuta* L

S.N.	Microorganism	Reference culture	Test extract (*T. minuta* L. essential oil)	ZOI of Solvent (mm)	Positive control		
			Zone of Inhibition (ZOI) (mm)		Name	Conc. (mcg)	ZOI (mm)
	Bacteria						
1.	*Bacillus subtilis*	ATCC 6051	0.00	0.00	Ciprofloxacin	5	31.16
2.	*Enterococcus faecalis*	ATCC 29212	0.00	0.00	Ciprofloxacin	5	21.40
3.	*Escherichia coli*	ATCC 8739	0.00	0.00	Ciprofloxacin	5	32.38
4.	*Klebsiella quasipneumoniae*	ATCC 700603	0.00	0.00	Ciprofloxacin	5	23.60
5.	*Proteus vulgaris*	ATCC 6380	10.50	0.00	Ciprofloxacin	5	32.06
6.	*Pseudomonas aeruginosa*	ATCC 9027	0.00	0.00	Ciprofloxacin	5	30.28
7.	*Salmonella enterica* subsp. *enterica* pv Typhi	Clinical Sample	0.00	0.00	Ciprofloxacin	5	34.60
8.	*Shigella dysenteriae*	Clinical Sample	0.00	0.00	Ciprofloxacin	5	33.72
9.	*Staphylococcus aureus*	ATCC 6538P	12.30	0.00	Amoxycillin	10	35.10
10.	*Staphylococcus epidermidis*	ATCC 12228	11.20	0.00	Amoxycillin	10	17.74
	Fungi						
1.	*Candida albicans*	ATCC 10231	0.00	0.00	Clotrimazole	10	16.20

flavonoids, phenolic, alkaloids which are often most associated with the antimicrobial activity (Verma et al., 2024). A key study isolated 19 acylated flavonol glycosides all from butanol and ethylacetate extracts that showed significant antibacterial activity. In contrast, the hexane fraction did not contain these active flavonols (Shahzadi & Shah, 2015). The ethylacetate extract revealed noticeable activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* with ZOI of 10.10 mm, 14.30 mm, and 17.50 mm respectively. Similarly, the methanolic extract showed a promising activity against *Proteus vulgaris, Staphylococcus aureus*, *Staphylococcus epidermidis* with ZOI of 9.80 mm, 10.40 mm and 16.30 mm respectively. Pillai et al. (2020) investigated the antimicrobial activity of *T. minuta* stem bark extracts obtained via maceration using four different solvents: hexane, ethylacetate, methanol and chloroform. Using the hole-plate diffusion method, they tested the extracts against six bacterial isolates viz. *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* (wild), *Escherichia coli* (O157:H7), *Pseudomonas aeruginosa* and *Serratia marcescens* and two fungal

Table 3: Antimicrobial activity test of Soxhlet extracts of *T. minuta* L. aerial part

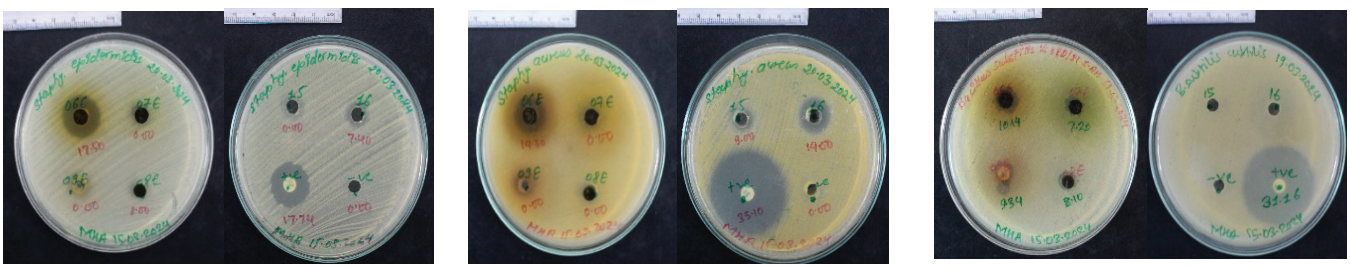
S.N.	Microorganism	Soxhlet extracts of *T. minuta* L. whole shoot plant	Zone of Inhibition (ZOI) (mm)	ZOI of Solvent (mm)	Positive control		
					Name	Conc. (mcg)	ZOI (mm)
	Bacteria						
1.	*Bacillus subtilis*	Hexane	0.00	0.00	Ciprofloxacin	5	31.16
		Ethyl Acetate	10.10				
		Methanol	0.00				
2.	*Enterococcus faecalis*	Hexane	0.00	0.00	Ciprofloxacin	5	21.40
		Ethyl Acetate					
		Methanol					
3.	*Escherichia coli*	Hexane	0.00	0.00	Ciprofloxacin	5	32.38
		Ethyl Acetate					
		Methanol					
4.	*Klebsiella quasipneumoniae*	Hexane	0.00	0.00	Ciprofloxacin	5	23.60
		Ethyl Acetate					
		Methanol					
5.	*Proteus vulgaris*	Hexane	0.00	0.00	Ciprofloxacin	5	32.06
		Ethyl Acetate	0.00				
		Methanol	9.50				
6.	*Pseudomonas aeruginosa*	Hexane	0.00	0.00	Ciprofloxacin	5	30.28
		Ethyl Acetate					
		Methanol					
7.	*Salmonella enterica* subsp. *enterica* pv Typhi	Hexane	0.00	0.00	Ciprofloxacin	5	34.60
		Ethyl Acetate					
		Methanol					
8.	*Shigella dysenteriae*	Hexane	0.00	0.00	Ciprofloxacin	5	33.72
		Ethyl Acetate					
		Methanol					
9.	*Staphylococcus aureus*	Hexane	0.00	0.00	Amoxycillin	10	35.10
		Ethyl Acetate	14.30				
		Methanol	10.40				
10.	*Staphylococcus epidermidis*	Hexane	0.00	0.00	Amoxycillin	10	17.74
		Ethyl Acetate	17.50				
		Methanol	16.30				
	Fungi						
1.	*Candida albicans*	Hexane	0.00	0.00	Clotrimazole	10	16.20
		Ethyl Acetate					
		Methanol					

isolates viz. *Candida albicans* and *Penicillium digitatum*. The inhibition zones were found to be in the ranges of 10.0±1.6 to 15.5±1.9 mm against bacterial isolates and 11.3±2.1 to 13.4±1.2 mm against *P. digitatum* but no activity was observed against *C. albicans* aligning the result of the present study. Therefore, *T. minuta*, being a very valuable plant, may also be useful for the treatment of diseases caused by different human pathogens. Such as: boils, blisters and redness of skin due to skin and soft skin infections by *S. Aureus* (Tong et al., 2015); sinus infection, endocarditis, intravascular catheter infections, cardiac devices, prosthetic joints, and CNS shunt infection by *S. epidermidis* (Lee & Anjum, 2024); urinary tract infection, wound infection, skin infection and respiratory tract infection caused by *P. vulgaris* (Kim et al., 2003) and infection of the blood, heart, lung, bone, eye, and brain infections by *B. subtilis* (Tokano et al., 2023), among others.

A picture showing positive zone of inhibition of *T. minuta* EO and its extracts (ethylacetate and methanolic) against different pathogens along with its positive and negative controls are depicted in Figure 2, Figure 3 and Figure 4 for further broader analysis.

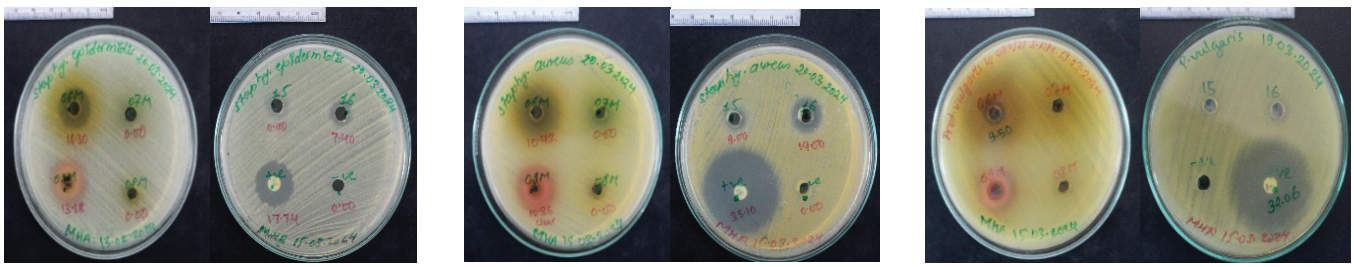
a. b. c.

Figure 2: Inhibition zones of *T. minuta* L. essential oil i.e. sample no. 14 against pathogenic strains, (**a.** *Staphylococus epidermidis,* **b.** *Staphylococcus aureus*, **c.** *Proteus vulgaris*) with their adjoining positive and negative controls



a. b. c.

Figure 3: Inhibition zones of *T. minuta* L. ethylacetate extract i.e. 06E against pathogenic strains (**a.** *Staphylococus epidermidis*, **b**. *Staphylococcus aureus*, **c.** *Bacillus subtilis*) with their adjoining positive and negative controls



a. b. c.

Figure 4: Inhibition zones of *T. minuta* L. methanolic extract i.e. 06M against pathogenic strains (**a.** *Staphylococus epidermidis*, **b.** *Staphylococcus aureus*, **c.** *Proteus vulgaris*) with their adjoining positive and negative controls

Conclusion

The essential oil extracted from *T. minuta* L. by the hydro-distillation method and analyzed by GC-MS demonstrated a rich chemical profile, with various other bioactive compounds. These constituents highlight the potential application plant in the pharmaceutical, agricultural, food, and perfumery industries. In antibacterial screening, the oil exhibited satisfactory inhibition zones with different bacterial strains most efficiently with gram-positive bacteria, suggesting it as a promising agent for further medicinal development. In addition to, the plant extracts show potential for antibacterial drug discovery and support ethno-pharmacological use and commercialization. But, in order to enlighten more precisely, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) can be suggested furthermore. This study confirms *T. minuta* as a naturally available, cheap, safe, and effective alternative to chemical bactericides. Finally, in the context of Nepal, *Tagetes minuta*, a potential medicinal herb, should be promoted for commercial cultivation, and stakeholders in the Nepalese business community should explore its market potential with continuous efforts and initiatives.

Author contributions

S. Adhikari did sample collection, Soxhlet extraction, antimicrobial analysis and manuscript preparation and S. Aryal also assisted during sample collection and also essential oil extraction, GC-MS analysis and Data recording.

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